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(54) Title: PEPTIDE MIXTURE AND VACCINE AGAINST A CHRONIC VIRAL INFECTION

(57) Abstract: Disclosed is a peptide mixture, a pharmaceutical composition and a vaccine against a chronic infection caused by a virus comprising a mixture of 10 to 30 amino acids (aa) long peptides each with a 5 to 25 aa overlap of the adjacent overlapping peptide spanning the amino acid sequence of a viral protein of said virus, e.g. Hepatitis B, Hepatitis C, GB virus-C, HIV and Herpes viruses. Hepatitis B has been used as an example and it is demonstrated that a peptide mixture composed of seventeen 20 to 23 aa long peptides spanning the amino acids 1 to 183 of the hepatitis B core antigen (HBcAg) could activate specific T cells regardless of the host MHC/HLA genotype that recognize the native protein processed by professional antigen presenting cells (APCs). Further, a method is described for the treatment of a viral infection, particularly a non-resolving chronic viral infection, making use of the novel peptide mixture immunogen.

Peptide mixture and vaccine against a chronic viral infection.

The present invention relates to a peptide mixture and vaccine against a chronic infection caused by a virus, such as hepatitis B, hepatitis C, GB virus-C, HIV and Herpes viruses. The peptide mixture is a multiple peptide T helper cell immunogen comprising overlapping peptides spanning the amino acid sequence of a viral protein of the infecting virus. The peptide mixture or the vaccine is useful for the treatment of the chronic infection in a patient.

Background of the invention

The CD4+ T helper cell response has been found to be essential in controlling the infection in several chronic viral diseases. Examples are the hepatitis B virus (HBV; Jung M.C. et al., Activation of a heterogeneous hepatitis B (HB) core and e antigen-specific CD4+ T-cell population during seroconversion to anti-HBe and anti-HBs in hepatitis B virus infection. J Virol 1995;69:3358-3368), the hepatitis C virus (HCV; Diepolder, H. M. et al, Possible mechanism involving T-lymphocyte response to non-structural protein 3 in viral clearance in acute hepatitis C virus infection. Lancet. 1995; 346(8981):1006-7; and Missale, G. et al, Different Clinical Behaviors Of Acute Hepatitis C Virus Infection Are Associated With Different Vigor Of the Anti-Viral Cell-Mediated Immune Response. J. Clin. Invest. 1996;98(3):706-714.), and the human immunodeficiency virus type 1 (HIV-1; Rosenberg, E. S. et al, Vigorous Hiv-1-Specific CD4(+) T Cell Responses Associated With Control Of Viremia.Science.1997; 278:1447-1450). However, for these three viruses different viral proteins seem to be the most important in priming the beneficial responses.

The problems with chronic viral infections are here exemplified by studying the hepatitis B virus (HBV). HBV is spread globally and today around 300-350 million persons are infected by HBV. HBV infection is a well documented cause for many types of liver injuries, including cirrhosis and hepatocellular carcinoma (HCC). The major routes for HBV transmission are vertical and sexual. When infected vertically, the infant has a 95% chance of developing a chronic infection whereas, when infected as an adult, the chance for chronicity is less than 5%. Thus, the HBV lifecycle has effectively adopted to the human host by means of vertical transmission. The effectiveness of this transmission route is most likely explained by the ability of the secretory hepatitis B virus e-antigen (HBeAg) to pass the placenta whereby an immunological tolerance is induced to both the HBV core (HBcAg) and HBeAg. This has been well documented in the transgenic mouse models. Concordantly, in humans the

observation that vertical transmission of HBV strains with a stop codon in the pre-core gene, thus making them unable to produce HBeAg, can cause fulminant hepatitis in the infant.

Clear-cut differences in the immune responses to HBV surface antigen (HBsAg), HBcAg and HBeAg have been documented in acute and chronic HBV infections. HBsAg-specific immune responses are highly effective in preventing HBV infections. Vaccines of today are based on the HBsAg and are effective in preventing new HBV infections in both neonates and adults. In acute and resolving chronic HBV infections activation of HBcAg- and HBeAg-specific, but not HBsAg-specific, CD4+ T cells have been found. These responses are almost never seen during non-resolving chronic HBV infections. Thus, the HBcAg- and HBeAg-specific CD4+ T cell response, in contrast to the HBsAg-specific response, plays a pivotal role in the clearance of HBV infections.

Nucleoside analogues have recently been introduced as an effective means to reduce the viral load in patients with chronic HBV infections. Almost all treated patients relapse after treatment stop with respect to both liver enzymes and the viral load. In contrast, around 30% of the patients treated with γ-interferon (γ-IFN) show a permanent response to HBV. Simultaneous with the γ-IFN induced clearance a HBcAg- and HBeAg-specific CD4+ T cell response can be detected. Thus, although nucleoside analogue therapy may be effective in transiently reducing the viral replication additional means to activate the endogenous immune response to HBV proteins are needed to increase the sustained response rate.

It is well known that synthetic peptides corresponding to viral T cell sites can prime T cells in vivo that recognize a viral protein processed by professional antigen presenting cells (APCs). APCs engulf the viral protein and digest it to short peptides which are associated with the MHC/HLA class II molecules of the host.

Several peptides derived from HBcAg have been found to be recognized by specific T cells in murine models. For example, the peptide 120-131 primes H-2^s restricted T cells, peptide 129-140 primes H-2^b restricted T cells, the peptide 85-96 primes H-2^d restricted T cells, and the peptide 100-120 primes H-2^f restricted T cells (Milich, Immunology Today 9:380-386, 1988). Also, immunization of mice with the respective peptide primes T cells that recognize HBcAg digested by APCs. Thus, these peptides represent true T cell sites. No similar immunisation experiments have been performed in humans. However, several peptides (1-25 and 61-85) derived from HBcAg have been found to be recognized by T cells from HBV infected humans (Jung et al., J Virol 69:3358-3368).

The determining factor for which peptides are to be presented by the host is determined by the MHC/HLA genotype of the host. Each MHC/HLA molecule can only bind

and present one or a few peptides from each viral protein. Subsequently, a T cell vaccine based on one single peptide is limited to only one or a few HLA classes.

Therefore, a T cell immunogen or vaccine that would activate virus-specific T cells regardless of the host HLA genotype would be universally useful and most desirable, particularly for the treatment of non-resolving chronic virus infections.

Description of the invention

The present invention is based on a multiple peptide T helper cell immunogen containing a complete viral protein in the form of overlapping synthetic peptides. The length of the sequences binding to different HLA class II molecules varies from 10-15 amino acids. Thus, by making the complete protein as 10 to 30 aa long peptides with 5 to 25 aa overlap between each peptide all possible 10-15 aa long peptides within the protein are represented.

Thus, the present invention is directed to a vaccine against a vaccine against a chronic infection caused by a virus comprising a mixture of 10 to 30 amino acids (aa) long peptides each with a 5 to 25 aa overlap of the adjacent overlapping peptide spanning the amino acid sequence of a viral protein of said virus, and a vehicle.

The vehicle to be used is selected by the vaccine manufacturer from vehicles accepted for use in human medicaments, and suitable candidates for the selection are provided in e.g. the European or US Pharmacopoeia.

The invention is also directed to a peptide mixture comprising 10 to 30 amino acids (aa) long peptides each with a 5 to 25 aa overlap of the adjacent overlapping peptide spanning the whole amino acid sequence of a viral protein of a virus causing chronic infections.

In preferred embodiments of the peptide mixture and the vaccine of the invention the virus is selected from Hepatitis B, Hepatitis C, GB virus-C, HIV and Herpes viruses, and the viral protein is selected from proteins comprising conserved regions. For example, conserved regions are found in hepatitis B core antigen, hepatitis C core antigen and enzyme, GB virus-C enzyme and envelope protein and HIV I core antigens p24 and p17.

In a further preferred embodiment of the peptide mixture and the vaccine of the invention the virus is Hepatitis B, and the viral protein is the hepatitis B core antigen.

In a more specific embodiment of the peptide mixture and the vaccine of the invention the mixture of peptides consists of 15 to 25 amino acids (aa) long peptides each with a 10 to 15 aa overlap of the adjacent overlapping peptide spanning the amino acids 1 to 183 of the hepatitis B core antigen (HBcAg).

In a most preferred embodiment of the peptide mixture and the vaccine of the invention the mixture is composed of seventeen 20 to 23 aa long peptides spanning the amino acids 1 to 183 of the hepatitis B core antigen (HBcAg), e.g. seventeen peptides having the amino acid sequences SEQ ID NO: 1 to 17 disclosed in the Sequence listing and in Table 1.

The invention is further directed to a peptide mixture according to the invention for use as a medicament.

The invention is additionally directed to a pharmaceutical composition comprising a peptide mixture according to the invention and a pharmaceutically acceptable carrier and/or diluent.

The pharmaceutically acceptable carrier and/or diluent to be used are/is selected from carriers and/or diluents known to be acceptable for human use, and suitable candidates for the selection are provided in e.g. the European or US Pharmacopoeia.

The invention is also directed to a method of treating a chronic infection caused by a virus in a patient comprising administering to the patient one or several immunologically effective dosages of a vaccine according to the invention or a peptide mixture according to the invention.

In a specific embodiment of the method of the invention the virus is Hepatitis B

It should be understood that the vaccine, the peptide mixture, or the pharmaceutical composition according to the invention may be used together with other antiviral compounds as combination therapy.

The invention will now be illustrated by reference to a more detailed description of embodiments, but these embodiments should not be considered as limiting to the scope of protection defined in the appended claims.

Synthesis of peptides

A total of 18 synthetic peptides, 20 (and 23) aa long with a 10 aa overlapp (See Table 1) corresponding to the aa sequence of HBcAg sybtype ayw (Galibert et al., 1979, Nature 281:646-648) were synthesized by standard techniques (Sällberg et al., 1991, Immunology Letters 30: 59-68) using an automated synthesizer (Syro, MyltiSynTech, Germany).

Preparation of the immunogens and immunizations

A total of one mg of each peptide was dissolved in one ml PBS. Each peptide was then added to a 100 µl mixture finally containing 5 µg of each peptide. The mixture was then emulsified in an equal volume of Freund's complete adjuvant. The final volume of 200 µl was then injected intra peritoneally into a mouse.

In vitro recall assays

Murine proliferation assays were performed as described previously [Sällberg, 1997 #152; Zhang, 1997 #151]. In brief, 50 µg recombinant protein was emulsified 1:1 in CFA was injected at 100 µl doses in groups of 5 -10 mice at the base of the tail. The mice were sacrificed 10 days later and draining lymphnodes were removed. Single cell suspensions were prepared in Clicks media and plated on microtiter plates at 6 x 10^5 cells per well. Recombinant protein was added in serial dilutions. Supernatant was taken after 24 hours for IL-2 and after 48 hours for IL-4, IL-5, IL-6 and γ-IFN. Cytokine concentrations were measured by EIA according to the manual (Endogen, Cambridge, MA). After 72 hours 3H-labeled thymidine (TdR; Amersham) was added, 16 hours later the labeled cells were harvested onto cellulose filters, quenched and the level of 3H-thymidine ([3H]TdR) uptake was determined by liquid scintillation using a beta-counter.

Results

Groups of five mice were immunized with 200 µg of the peptide mixture in adjuvant as described. Ten days later the mice were sacrificed, spleens were removed and recall cultures were set. Immunization with the peptide mixture clearly primed H-2b restricted T cells which recognize the previously described T cell site at residues 129-140 previously described (Milich, Immunology Today 9:380-386, 1988). Importantly, the peptide mixture primes T cells which recognize the peptides generated by APC processing and presentation of native like recombinant HBcAg (kindly provided by Dr Darrell Peterson, Commonwealth University, VA). Thus, despite the fact that the peptides are in no way optimized with respect to size for interaction with the $H\text{-}2^b$ molecule they can effectively prime specific T cells. Primed T cells show the same specificity with respect to both IL-2 and γIFN production suggesting that the same T cell population produces both cytokines (See Table 2).

In conclusion, by immunization with a mixture of overlapping peptides spanning a complete protein sequence specific T cells can be activated regardless of the host MHC/HLA genotype that recognize the native protein processed by professional antigen presenting cells (APCs).

Table 1. Peptide sequences included in the immunogen mixture. The sequence corresponds to HBcAg of subtype ayw (Galibert et al., 1979).

Residues	Peptide sequence	SEQ ID NO
1-20	MDIDPYKEFGATVELLSFLP	SEQ ID NO: 1
11-30	ATVELLSFLPSDFFPSVRDL	SEQ ID NO: 2
21-40	SDFFPSVRDLLDTASALYRE	SEQ ID NO: 3
31-50	LDTASALYREALESPEHCSP	SEQ ID NO: 4
41-60	ALESPEHCSPHHTALRQAIL	SEQ ID NO: 5
51-70	HHTALRQAILCWGELMTLAT	SEQ ID NO: 6
61-80	CWGELMTLATWVGVNLEDPA	SEQ ID NO: 7
71-90	WVGVNLEDPASRDLVVSYVN	SEQ ID NO: 8
81-100	SRDLVVSYVNTNMGLKFRQL	SEQ ID NO: 9
91-110	TNMGLKFRQLLWFHISCLTF	SEQ ID NO: 10
101-120	LWFHISCLTFGRETVIEYLV	SEQ ID NO: 11
111-130	GRETVIEYLVSFGVWIRTPP	SEQ ID NO: 12
121-140	SFGVWIRTPPAYRPPNAPIL	SEQ ID NO: 13
131-150	AYRPPNAPILSTLPETTVVR	SEQ ID NO: 14
141-160	STLPETTVVRRRGRSPRRRT	SEQ ID NO: 15
151-170	RRGRSPRRRTPSPRRRRSQS	SEQ ID NO: 16
161-183	PSPRRRRSQSPRRRRSQSRESQC	SEQ ID NO: 17

Table 2. In vitro recall of lymphnode T cell from mice immunized with the peptide mixture containing 5µg each of the peptides SEQ ID NO: 1 to 17.

Recall Antigen	Amount µg/ml (peptide no)	Amount in vitro recalled cytokine (pg/ml) IL-2	γIFN	Amount NKA proliferation (Δcpm)
HBcAg	20	<15	620	7526
HBcAg	4	42	8656	11859
HBcAg	0.8	23	7259	4915
HBcAg	0.16	15	3345	2255
HBcAg	0.032	<15	1136	875
HBcAg	0.0064	17	0	283
HBcAg	0.00128	<15	0	496
PHA	1	821	11070	30231
Media	-	<15	0	0
Media	-	<15	0	0
1-20	(1)	20	0	3552
11-30	(2)	<15	0	248
21-40	(3)	26	0	0
31-50	(4)	30	0	0
41-60	(5)	75	351	5723
51-70	(6)	15	0	0
61-80	(7)	<15	0	0
71-90	(8)	23	0	0
81-100	(9)	29	0	2648
91-110	(10)	<15	0	347
101-120	(11)	<15	0	0
111-130	(12)	43	0	10712
121-140	(13)	237	11013	30923
131-150	(14)	35	1286	16434
141-160	(15)	<15	0	0
151-170	(16)	<15	0	0
161-183	(17)	<15	0	96

Abbrevations:

NKA = a cell line whose proliferation is proportional to the presence of IL-2 in the culture supernatant. Thus, a sensitive indicator for the presence of IL-2.

Δcpm = the [3H] thymidine uptake determined as counts per minute (cpm) with addition of recall antigens minus the cpm with media alone.

Claims

1. Vaccine against a chronic infection caused by a virus comprising a mixture of 10 to 30 amino acids (aa) long peptides each with a 5 to 25 aa overlap of the adjacent overlapping peptide spanning the amino acid sequence of a viral protein of said virus, and a vehicle.

2. Vaccine according to claim 1, wherein the virus is selected from Hepatitis B, Hepatitis C, GB virus-C, HIV and Herpes viruses, and the viral protein is selected from proteins comprising conserved regions.

3. Vaccine according to claim 2, wherein the virus is Hepatitis B, and the viral protein is the hepatitis B core antigen.

4. Vaccine according to claim 3, wherein the mixture of peptides consists of 15 to 25 amino acids (aa) long peptides each with a 10 to 15 aa overlap of the adjacent overlapping peptide spanning the amino acids 1 to 183 of the hepatitis B core antigen (HBcAg).

5. Vaccine according to claim 1, wherein the mixture is composed of seventeen 20 to 23 aa long peptides spanning the amino acids 1 to 183 of the hepatitis B core antigen (HBcAg).

6. Vaccine according to claim 5, wherein the mixture is composed of seventeen peptides having the amino acid sequences SEQ ID NO: 1 to 17.

7. Peptide mixture comprising 10 to 30 amino acids (aa) long peptides each with a 5 to 25 aa overlap of the adjacent overlapping peptide spanning the whole amino acid sequence of a viral protein of a virus causing chronic infections.

8. Peptide mixture according to claim 7, wherein the virus is selected from Hepatitis B, Hepatitis C, GB virus-C, HIV and Herpes viruses, and the viral protein is selected from proteins comprising conserved regions.

9. Peptide mixture according to claim 8, wherein the virus is Hepatitis B, and the viral protein is the hepatitis B core antigen.

10. Peptide mixture according to claim 9, wherein the mixture of peptides consists of 15 to 25 amino acids (aa) long peptides each with a 10 to 15 aa overlap of the adjacent overlapping peptide spanning the amino acids 1 to 183 of the hepatitis B core antigen (HBcAg).

11. Peptide mixture according to claim 1, wherein the mixture is composed of seventeen 20 to 23 aa long peptides spanning the amino acids 1 to 183 of the hepatitis B core antigen (HBcAg).

12. Peptide mixture according to claim 11, wherein the mixture is composed of seventeen peptides having the amino acid sequences SEQ ID NO: 1 to 17.

13. Peptide mixture according to any one of claims 7-12 for use as a medicament.

14. Pharmaceutical composition comprising a peptide mixture according to any one of claims 7 - 12 and a pharmaceutically acceptable carrier and/or diluent.

15. Method of treating a chronic infection caused by a virus in a patient comprising administering to the patient one or several immunologically effective dosages of a vaccine according to any one of claims 1 - 6 or a peptide mixture according to any one of claims 7 - 12.

16. Method of treating a chronic infection caused by a virus according to claim 15, wherein the virus is Hepatitis B and the vaccine is according to any one of claims 4 - 6, and the peptide mixture is according to any one of claims 9 - 12.

SEQUENCE LISTING

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